"Contains NO CBI"

(1)

UNION CARBIDE CORPORATION 39 OLD RIDGEBURY ROAD, DANBURY, CT 06817-0001

8EHQ-92-12832 (A)
8892001097

August 21, 1992

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Document Processing Center (TS-790)
Room L-100
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

92 AUG 28 PM 2:39

Attn: Section 8(e) Coordinator (CAP Agreement)

Re: CAP Agreement Identification No. 8ECAP-0110

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith lists the following reports pursuant to the terms of the TSCA §8(e) Compliance Audit Program and Union Carbide's CAP Agreement dated August 14, 1991 (8ECAP-0110). These reports describe mutagenicity studies with Silane A-186 (CASRN 3388-04-3).

1. "Mutagenicity Evaluation of CHF-41-14 in the Mouse Lymphoma Forward Mutation Assay", Litton Bionetics, LBI Project No. 20839, June 1, 1978.

2. "Mutagenicity Evaluation of CHF-41-14 in the Unscheduled DNA Synthesis in Human WI-38 Cells Assay", Litton Bionetics, LBI Project No. 20840, July 1, 1978.

3. "Mutagenicity Evaluation of CHF-41-14 in the sister Chromatid Exchange Assay in L5178Y Mouse Lymphoma Cells", Litton Bionetics, LBI Project No. 20990, March 1, 1979.

4. "Mutagenicity Evaluation of CHF-40-321", LItton Bionetics, LBI Project No. 20838, November 1, 1977.

Complete summaries of these reports are attached.

CHF-4114

RECEIVED 9/29/94

(2)

This information was previously submitted to the Agency in the following manner:

UCC letters to EPA's 8(e) Office [8EHQ-0681-0402]

Additional copies of these studies are attached.

Complete summaries of these reports are attached.

Previous TSCA Section 8(e) or "FYI" Submission(s) related to this substance are:

8EHQ-0681-0402

Previous PMN submissions related to this substance are: (None)

This information is submitted in light of EPA's current guidance. Union Carbide does not necessarily agree that this information reasonably supports the conclusion that the subject chemical presents a substantial risk of injury to health or the environment.

In the attached reports the term "Confidential" may appear. This precautionary statement was for internal use at the time of issuance of these reports. Confidentiality is hereby waived for purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that the publication rights to the contained information are the property of Union Carbide.

Yours truly,

William C. Kuryla, Ph.D.
Associate Director
Product Safety
(203/794-5230)

WCK/cr
Attachment (3 copies of cover letter, summaries, and reports)

SUMMARY

MUTAGENICITY EVALUATION

OF

CHF-40-321 (Ames)

FINAL REPORT

A-186

SUBMITTED TO

CARNEGIE-MELLON INSTITUTE OF RESEARCH
4400 FIFTH AVENUE
PITTSBURGH, PENNSYLVANIA 15213

SUBMITTED BY

LITTON BIONETICS, INCORPORATED
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

NOVEMBER, 1977

SUMMARY

Conclusions

The test compound, CHF-40-321, exhibited mutagenic activity with the strains TA-1535 and TA-100 in the activation and nonactivaiton assays conducted in this evaluation and is considered as mutagenic under these test conditions. These tests indicate that the test compound predominantly induces missense mutations and it does not require metabolic activation to be genetically active.

SUMMARY

MUTAGENICITY EVALUATION OF

CHF-41-14 IC - A186

IN THE
SISTER CHROMATID EXCHANGE ASSAY
IN L5178Y MOUSE LYMPHOMA CELLS

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON
4400 FIFTH AVE.
PITTSBURGH, PA. 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO: 20990

MARCH 1979
1

SUMMARY

INTERPRETATION OF RESULTS:

The test compound, CHF-41-14, was evaluated for its ability to induce SCEs directly and also in the presence of a metabolic activation system containing liver microsomal enzymes from Aroclor-induced rats.

Stock solution of the test compound was prepared in DMSO at 125 µl/ml and serial dilutions were performed, using the same solvent, so that the final concentration of solution did not exceed 0.2 ml per culture tube (0.02 ml/ml) at the predetermined dose levels. Typically the concentration was 0.1 ml per tube or less, and so 0.1 ml of DMSO was added to the solvent control cultures.

When used directly the compound proved to be toxic between 1.25 and 2.00 µl/ml, to the extent that progression through the mitotic cycle was inhibited so that second division metaphase cells were not present in the harvest (Table 1). The two lowest dose levels employed in this study did yield results, however, with 0.625 µl/ml inducing a significant increase in SCE frequency.

When tested with the activation system, CHF-41-14 was far more toxic than when used directly and was therefore used at much lower doses (Table 2). All but the lowest dose induced significant increases in SCE frequency. The solvent control frequency is abnormally high (14.7 SCE/Cell as opposed to the typical 9-12), but the ability of this compound to induce SCE is nonetheless clear because SCE frequencies increase consistently with dose.

While the positive results obtained with activation were unequivocal, the nonactivation results were more difficult to interpret because only two dose levels yielded data. Therefore, a second study was conducted using lower doses for the nonactivation series to ensure the harvest of second division metaphase cells.

Results of this second nonactivation study, presented in Table 3, indicate that the highest concentration (0.16 µl/ml) induced a significant increase in SCE frequency. At lower doses the frequenices were within normal control range. These results, considered together with those of the initial study, show that CHF-41-14 may induce significant increases in SCE frequency at concentrations in excess of 0.08 µl/ml, and suggest a positive but erratic dose response.

In the repeat test (Study 2) with activation, (Table 4), the doses were identical to those employed in the first study, the control SCE frequencies were quite normal, and the highest dose (0.04 µl/ml) once again caused a two-fold increase in SCE frequency (compared to solvent control). Study 2 differs from Study 1 in that 1) frequencies at lower doses were elevated, but not significantly ($P > 0.01$); 2) the tendency to increase with dose is poorly defined; and 3) frequencies were 30-50% lower at all dose levels as well as in the solvent control. It seems clear, nevertheless, that the test compound does induce SCEs and that SCE frequencies tend to increase with dose.

The upward, overall shift in SCE frequency in the first study cannot be adequately explained, but it is presumably due to the solvent (DMSO) because the negative control frequency was within normal range. Perhaps the DMSO contained an SCE-inducing impurity; or perhaps it inhibited cell progression through the mitotic cycle so that the effective BrdU/cell ratio was increased, resulting in an overall increase in SCE frequency (see Stetka and Carrano, Chromosoma 63, 21-31, 1977).
A different lot of DMSO was used in the second study, and the first lot is no longer available, so these hypotheses cannot be tested. It is not felt that these unusual circumstances should in any way lower confidence in the overall conclusions drawn from these data.

CONCLUSIONS:

Used directly, CHF-41-14 induces small increases in SCE frequency at concentrations in excess of 0.08 µl/ml. When used together with the metabolic activation system the compound becomes far more toxic and far more potent in terms of SCE induction. Thus, CHF-41-14 does induce SCEs under the conditions of this assay.

SUMMARY

MUTAGENICITY EVALUATION OF

CHF-41-14 - UCC A-186

IN THE
UNSCHEDULED DNA SYNTHESIS IN
HUMAN WI-38 CELLS ASSAY

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON INSTITUTE OF RESEARCH
4400 FIFTH AVENUE
PITTSBURGH, PENNSYLVANIA 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20840

JULY 1978

SUMMARY

INTERPRETATION OF RESULTS AND CONCLUSIONS

The test material, CHF-41-14, was prepared as a stock solution in DMSO at 50 µl/ml. The material was soluble in the stock solution, but formed a precipitate when added to the tissue culture medium during the assay. The compound was tested for its toxicity in WI-38 cells and based on these results, a series of concentrations were selected for the actual test. The concentrations employed in activation (+S9 mix) and nonactivation (-S9 mix) assays ranged from 0.00125 µl/ml to 0.01 µl/ml.

The results of the study are shown in Tables 1 and 2. The data from the test run with mouse liver S9 mix were negative. All test values were near the control value. The nonactivation test run showed a single value of 165% of the control. This value was considered significantly higher than the control. However, all other concentrations were below a level considered significant and no clear dose response was observed. The results may represent a narrow range of activity around 0.0025 µl/ml, but the lack of any significant toxicity at 0.005 and 0.01 µl reduces the chances that the drop in activity is due to cell death.

CHF-41-14 appeared to show weak activity in this assay. The data suggest some induction of DNA damage at a concentration of 0.0025 µl/ml. The lack of a good dose response over the high end of the dose range and the complete absence of a response with mouse liver S9 mix places the weak response under some suspicion. The compound could be considered only weakly active at best.

SUMMARY

MUTAGENICITY EVALUATION OF

CHF-41-14 — UCC-A186

IN THE
MOUSE LYMPHOMA FORWARD
MUTATION ASSAY

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON
4400 FIFTH AVENUE
PITTSBURGH, PA 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20839

JUNE 1978

SUMMARY

The material was shown to be a mutagen in this assay and produced a dose related increase in mutation frequency under nonactivation (-S9) conditions. The data from the activation tests were also elevated at the higher concentration levels but the magnitude of the responses was much less than that observed without S9. This information could be viewed as an indication of quenching or detoxification by the S9 fraction.

The test material, CHF-41-14, was mutagenic in the Mouse Lymphoma Forward Mutation Assay. The mutagenic response was greater under nonactivation test conditions than activation test conditions.

AFDR

MUTAGENICITY EVALUATION

OF

CHF-40-321 (Ames)

FINAL REPORT

A-186

SUBMITTED TO

CARNEGIE-MELLON INSTITUTE OF RESEARCH
4400 FIFTH AVENUE
PITTSBURGH, PENNSYLVANIA 15213

SUBMITTED BY

LITTON BIONETICS, INCORPORATED
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

NOVEMBER, 1977

Litton BIONETICS

TABLE OF CONTENTS

SPONSOR: Carnegie-Mellon Institute of Research

MATERIAL: CHF-40-321

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: September 12, 1977

2. Description: Colorless liquid

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

Component	Final Concentration/ml
TPN	4 µmoles
Glucose-6-phosphate	5 µmoles
Sodium phosphate (dibasic)	100 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

ASSAY	CHEMICAL[a]	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS[b]
	2-Nitrofluorene (NF)	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard (QM)	Water or saline	FS[b]
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide[c]	BPS[b]
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide[c]	FS[b]

[a] Concentrations given in Results Section

[b] BPS = Base-pair substitution

FS = Frameshift

[c] Previously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

IV. RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: CHF-40-321
B. SOLVENT: DMSO
C. TEST INITIATION DATE: SEPT. 15, 1977
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		D4*	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL	---	---	21		16		24		28		115	136	27	
POSITIVE CONTROL**	---	---	938		711		>1000		>1000		>1000	-	944	
TEST COMPOUND														
0.001000 UL	---	---	24		25		21		22		102	-	24	
0.010000 UL	---	---	21		20		26		30		123	-	25	
0.100000 UL	---	---	28		24		21		29		134	-	11	
1.000000 UL	---	---	62		25		19		21		148	189	16	
5.000000 UL	---	---	117		26		12		29		219	278	15	
10.000000 UL	---	---	-		-		-		-		-	283	-	
20.000000 UL	---	---	-		-		-		-		-		-	
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	20	22	22		27		50		128	169	49	
POSITIVE CONTROL***	RAT	LIVER	238	-	317		>1000		>1000		655	-	98	
TEST COMPOUND														
0.001000 UL	RAT	LIVER	24	-	19		28		44		100	-	51	
0.010000 UL	RAT	LIVER	26	-	18		21		36		147	-	36	
0.100000 UL	RAT	LIVER	18	-	14		24		45		162		37	
1.000000 UL	RAT	LIVER	35	26	15		31		41		165	270	58	
5.000000 UL	RAT	LIVER	77	49	15		25		47		190	264	42	
10.000000 UL	RAT	LIVER	-	86	-		-		-		-	345	-	
20.000000 UL	RAT	LIVER	-	8	-		-		-		-	219	-	

* TRY+ CONVERTANTS PER PLATE

** TA-1535 MNNG 10 UG/PLATE
TA-1537 QM 10 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 10 UG/PLATE
D4 MNNG 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE
- INDICATES TEST WAS NOT DONE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE
D4 DMNA 100 MICROMOLES/PLATE
SOLVENT DMSO 50 UL/PLATE

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity Test Results

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 µl to 5 µl per plate.

B. Nonactivation Test Results

The compound was mutagenic to strains TA-1535 and TA-100. An additional test was performed with TA-100 employing a higher dose level of 10 µl per plate because of the increased mutation frequency at the high dose level in the initial test. The positive results was confirmed in this test.

C. Activation Test Results

The test compound was mutagenic to strains TA-1535 and TA-100. Additional tests were performed with these two strains, employing higher doses of 10 µl and 20 µl per plate because of the dose-related effect in the reversion frequencies. This positive effect was confirmed in the second tests.

5. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

D. Conclusions

The test compound, CHF-40-321, exhibited mutagenic activity with the strains TA-1535 and TA-100 in the activation and nonactivaiton assays conducted in this evaluation and is considered as mutagenic under these test conditions. These tests indicate that the test compound predominantly induces missense mutations and it does not require metabolic activation to be genetically active.

Submitted by:

D.R. Jagannath 11.22.77

D.R. Jagannath, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Molecular
Toxicology

Reviewed by:

David J. Brusick 11/22/77

David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.

- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.

- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.

- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

GENETICS ASSAY #2827

AFDR Bundle

MUTAGENICITY EVALUATION OF

CHF-41-14 IC - A186

IN THE
SISTER CHROMATID EXCHANGE ASSAY
IN L5178Y MOUSE LYMPHOMA CELLS

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON
4400 FIFTH AVE.
PITTSBURGH, PA. 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO: 20990

MARCH 1979

Litton BIONETICS

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-VIII. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study. Item VI identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VII. Item VIII provides the conclusion and evaluation.

The second part of the report, entitled PROTOCOL, describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices.

All test and control results presented in this report are supported by raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland, 20795.

Copies of the raw data will be supplied to the sponsor upon request. Copies of raw data are provided in the appendix.

I. SPONSOR: Carnegie-Mellon

II. MATERIAL TESTED

A. Client's Identification: CHF-41-14

B. Genetic's Assay No.: 2827

C. Date Received: February 7, 1978

D. Physical Description:

III. TYPE OF ASSAY: Sister Chromatid Exchange Assay

IV. PROTOCOL NO.: 433

V. STUDY DATES:

A. Initiation Date: December 13, 1978

B. Completion Date: February 6, 1979

VI. RESULTS

The results of this assay are presented in Tables 1-4.

VII. INTERPRETATION OF RESULTS:

The test compound, CHF-41-14, was evaluated for its ability to induce SCEs directly and also in the presence of a metabolic activation system containing liver microsomal enzymes from Aroclor-induced rats.

Stock solution of the test compound was prepared in DMSO at 125 µl/ml and serial dilutions were performed, using the same solvent, so that the final concentration of solution did not exceed 0.2 ml per culture tube (0.02 ml/ml) at the predetermined dose levels. Typically the concentration was 0.1 ml per tube or less, and so 0.1 ml of DMSO was added to the solvent control cultures.

When used directly the compound proved to be toxic between 1.25 and 2.00 µl/ml, to the extent that progression through the mitotic cycle was inhibited so that second division metaphase cells were not present in the harvest (Table 1). The two lowest dose levels employed in this study did yield results, however, with 0.625 µl/ml inducing a significant increase in SCE frequency.

When tested with the activation system, CHF-41-14 was far more toxic than when used directly and was therefore used at much lower doses (Table 2). All but the lowest dose induced significant increases in SCE frequency. The solvent control

VII. INTERPRETATION OF RESULTS: (continued)

frequency is abnormally high (14.7 SCE/Cell as opposed to the typical 9-12), but the ability of this compound to induce SCE is nonetheless clear because SCE frequencies increase consistently with dose.

While the positive results obtained with activation were unequivocal, the nonactivation results were more difficult to interpret because only two dose levels yielded data. Therefore, a second study was conducted using lower doses for the nonactivation series to ensure the harvest of second division metaphase cells.

Results of this second nonactivation study, presented in Table 3, indicate that the highest concentration (0.16 µl/ml) induced a significant increase in SCE frequency. At lower doses the frequenices were within normal control range. These results, considered together with those of the initial study, show that CHF-41-14 may induce significant increases in SCE frequency at concentrations in excess of 0.08 µl/ml, and suggest a positive but erratic dose response.

In the repeat test (Study 2) with activation, (Table 4), the doses were identical to those employed in the first study, the control SCE frequencies were quite normal, and the highest dose (0.04 µl/ml) once again caused a two-fold increase in SCE frequency (compared to solvent control). Study 2 differs from Study 1 in that 1) frequencies at lower doses were elevated, but not significantly ($P > 0.01$); 2) the tendency to increase with dose is poorly defined; and 3) frequencies were 30-50% lower at all dose levels as well as in the solvent control. It seems clear, nevertheless, that the test compound does induce SCEs and that SCE frequencies tend to increase with dose.

The upward, overall shift in SCE frequency in the first study cannot be adequately explained, but it is presumably due to the solvent (DMSO) because the negative control frequency was within normal range. Perhaps the DMSO contained an SCE-inducing impurity; or perhaps it inhibited cell progression through the mitotic cycle so that the effective BrdU/cell ratio was increased, resulting in an overall increase in SCE frequency (see Stetka and Carrano, Chromosoma 63, 21-31, 1977).
A different lot of DMSO was used in the second study, and the first lot is no longer available, so these hypotheses cannot be tested. It is not felt that these unusual circumstances should in any way lower confidence in the overall conclusions drawn from these data.

VIII. CONCLUSIONS:

Used directly, CHF-41-14 induces small increases in SCE frequency at concentrations in excess of 0.08 µl/ml. When used together with the metabolic activation system the compound becomes far more toxic and far more potent in terms of SCE induction. Thus, CHF-41-14 does induce SCEs under the conditions of this assay.

Submitted by:

Study Director

Daniel Stetka

Daniel Stetka, Ph.D.
Section Leader
Animal Genetics and
Cytogenetics
Department of Genetics
and Cell Biology

4/4/79
Date

Reviewed by:

David J. Brusick

David J. Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

4/5/79
Date

Litton BIONETICS

TABLE 1

SCEs IN MOUSE LYMPHOMA CELLS - NONACTIVATION (Study 1)

Treatment	Concentration	No. of SCEs per Cell	
		Mean	SD
Negative Control (medium)	--	11.7	4.9
Positive Control (EMS)	0.5 µl/ml	58.9	22.3*
Solvent Control (DMSO)	0.1 ml/tube	11.7	3.9
CHF-41-14	0.313 µl/ml	12.7	2.4
CHF-41-14	0.625 µl/ml	18.5	3.4*
CHF-41-14	1.250 µl/ml 1.500 µl/ml 1.750 µl/ml 2.000 µl/ml	No scorable, second division metaphase cells were obtained at these dose levels	

* Significantly greater than solvent control value, $P < 0.01$ (Student's t-test).

Data analyzed on the basis of number of SCEs per cell scored 10 cells scored for each treatment.

TABLE 2

SCEs IN MOUSE LYMPHOMA CELLS - ACTIVATION (STUDY 1)

Treatment	Concentration	No. of SCEs per Cell	
		Mean	SD
Negative Control (medium)	--	9.9	3.1
Positive Control (DMN)	0.3 µl/ml	48.8	13.0*
Solvent Control (DMSO)	0.1 ml/tube	14.7	3.5
CHF-41-14	0.0025 µl/ml	14.5	3.4
CHF-41-14	0.0050 µl/ml	20.7	3.6*
CHF-41-14	0.010 µl/ml	24.6	6.9*
CHF-41-14	0.020 µl/ml	26.6	8.0*
CHF-41-14	0.04 µl/ml	30.6	5.9*

* Significantly greater than solvent control value, $P < 0.01$ (Student's t-test).

Data analyzed on the basis of number of SCEs per cell scored
10 cells scored for each treatment.

TABLE 3

SCEs IN MOUSE LYMPHOMA CELLS -- NONACTIVATION (Study 2)

Treatment	Concentration	No. of SCEs per Cell	
		Mean	SD
Negative Control (medium)	--	8.9	3.2
Positive Control (EMS)	0.5 µl/ml	69.4	20.3*
Solvent Control (DMSO)	0.1 ml/tube	8.9	3.5
CHF-41-14	0.01 µl/ml	10.6	3.0
CHF-41-14	0.02 µl/ml	8.4	2.8
CHF-41-14	0.04 µl/ml	12.2	2.9
CHF-41-14	0.08 µl/ml	10.5	3.4
CHF-41-14	0.16 µl/ml	14.3	4.6*

* Significantly greater than solvent control value, $P < 0.01$ (Student's t-test).

Data analyzed on the basis of number of SCEs per cell scored 10 cells scored for each treatment.

TABLE 4

SCEs IN MOUSE LYMPHOMA CELLS - ACTIVATION (Study 2)

Treatment	Concentration	No. of SCEs per Cell	
		Mean	SD
Negative Control (medium)	--	11.0	2.7
Positive Control (DMN)	0.3 µl/ml	55.5	9.7*
Solvent Control (DMSO)	0.1 ml/flask	10.0	2.8
CHF-41-14	0.0025 µl/ml	12.2	2.0
CHF-41-14	0.0050 µl/ml	11.5	3.7
CHF-41-14	0.010 µl/ml	12.2	3.7
CHF-41-14	0.020 µl/ml	13.0	3.8
CHF-41-14	0.040 µl/ml	19.4	6.2*

* Significantly greater than solvent control value, $P < 0.01$ (Student's t-test).

Data analyzed on the basis of number of SCEs per cell scored 10 cells scored for each treatment.

PROTOCOL

1. OBJECTIVE

The objective of this study was to evaluate CHF-41-14 for Sister Chromatid Exchange (SCE) induction in L5178Y mouse lymphoma cells.

2. MATERIALS AND METHODS

A. Toxicity

The solubility, toxicity, and doses for all chemicals were determined prior to screening. The effect of each chemical on the survival of the indicator cells was determined by exposing the cells to a wide range of chemical concentrations in complete growth medium. Toxicity was measured as loss in growth potential of the cells induced by a four-hour exposure to the chemical followed by a 24-hour expression period in growth medium. Some toxic as well as several non-toxic doses were then selected for use in the initial study.

B. Indicator Cells

The cells used in this study were derived from Fischer mouse lymphoma cell line L5178Y. The cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BrdU) sensitive.

C. Media

The cells were maintained in Fischer's medium for leukemic cells of mice with 10% horse serum and sodium pyruvate.

D. Control Compounds

1. Negative Control

The solvent in which the test compound was prepared was used as the solvent or vehicle control and is designated as solvent control in the data table. The actual solvent is listed in Table 1 of Section V. Results. A negative control consisting of cells exposed to media only is also used in the assay.

2. MATERIALS AND METHODS (continued)

D. Control Compounds

2. Positive Control

Ethylmethanesulfonate (EMS), which induces mutation by base-pair substitution, was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of 0.5 µl/ml.

Dimethylnitrosamine (DMN), which induces mutation by base-pair substitution and requires metabolic biotransformation by microsomal enzymes, was used as a positive control substance for activation studies at a final concentration of 0.3 µl/ml.

E. Cell Treatment

Mouse lymphoma cells (L5178Y) were treated as described below. The test compound was added to aliquots of 3 million cells in growth medium at the predetermined doses with or without an S-9 activation mixture and incubated at 37°C for 4 hours on a rocker. The incubation period was terminated by washing the cells twice with growth medium. BrdU (0.1 mM final concentration) was then added to the culture tubes and incubation was continued in the dark for 20 hours or two cell cycles. This permits BrdU to be incorporated into the DNA through two replication cycles so that sister chromatid exchanges may be detected.

Colcemid was added to a concentration of 0.1 ug/ml during the last 3 hours of incubation, and metaphase cells collected by centrifugation. * Treated cells were harvested in 0.075 M KCL fixed in Carnoy's fixative and air-dried onto microscope slides.

Sister chromatid exchanges were visualized by staining with techniques described in Stetka et al (Mutat. Res. 51, 1978).

F. Activation System

1. S9 Mixture

Component	Final Concentration/ml
NADP (Sodium salt)	2.4 mg
Isocitric acid	4.5 mg
Homogenate S9 fraction	15 µl

* In the initial study, colchicine was employed at a final concentration of 5×10^{-5} mg/ml. Colcemid was used in the second study.

2. S9 Homogenate

A 9,000 x g supernatant was prepared from Fischer 344 adult male rat liver induced by Aroclor 1254 five days prior to kill according to the procedure of Ames et al. (1975). S9 samples were coded by lot number and assayed for milligrams protein per milliliter and relative P448/P450 activity by methods described in LBI Technical Data on Rat Liver S9 Product.

3. RESULTS

The data presented in Tables 1 - 4 show the concentrations of the test compound employed and the number of SCE's per cell.

Interpretation of data is based on the relative increase in SCE with respect to dose compared to the spontaneous level. Statistical analysis of the data is made by a t-statistic.

REFERENCES

Ames, B.N., McCann, J. and Yamasake, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/ mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

AFDR
Bundle w/
LBI 20839

MUTAGENICITY EVALUATION OF

CHF-41-14 - UCC A-186

IN THE UNSCHEDULED DNA SYNTHESIS IN HUMAN WI-38 CELLS ASSAY

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON INSTITUTE OF RESEARCH
4400 FIFTH AVENUE
PITTSBURGH, PENNSYLVANIA 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20840

JULY 1978

Litton BIONETICS

PREFACE

This report contains a summary of the data compiled during the evaluation of the test material. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IV and provides sponsor and compound identification information, type of assay and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V identifies the tables and/or figures containing the data used by the Study Director in interpreting the test results (item VI).

The second part of the report, entitled PROTOCOL, describes, in detail, the materials and procedures employed in conducting the evalution. This part of the report also contains evaluation criteria, standard operating procedures and any appendices. These are included to acquaint the sponsor with the methods used to develop and analyze the test results. All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology. Copies of raw data will be supplied to the sponsor upon request.

I. SPONSOR: Carnegie-Mellon

II. MATERIAL

A. Identification: CHF-41-14

B. Date Received: February 7, 1978

C. Physical Description: Pale yellow liquid

III. TYPE OF ASSAY: Unscheduled DNA Synthesis Assay

IV. PROTOCOL NO.: DMT-108

V. RESULTS

The test material was evaluated for its ability to induce unscheduled DNA synthesis in human WI-38 cells blocked in G_1 phase. The results are presented in Tables 1 and 2.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test material, CHF-41-14, was prepared as a stock solution in DMSO at 50 µl/ml. The material was soluble in the stock solution, but formed a precipitate when added to the tissue culture medium during the assay. The compound was tested for its toxicity in WI-38 cells and based on these results, a series of concentrations were selected for the actual test. The concentrations employed in activation (+S9 mix) and nonactivation (-S9 mix) assays ranged from 0.00125 µl/ml to 0.01 µl/ml.

The results of the study are shown in Tables 1 and 2. The data from the test run with mouse liver S9 mix were negative. All test values were near the control value. The nonactivation test run showed a single value of 165% of the control. This value was considered significantly higher than the control. However, all other concentrations were below a level considered significant and no clear dose response was observed. The results may represent a narrow range of activity around 0.0025 µl/ml, but the lack of any significant toxicity at 0.005 and 0.01 µl reduces the chances that the drop in activity is due to cell death.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

CHF-41-14 appeared to show weak activity in this assay. The data suggest some induction of DNA damage at a concentration of 0.0025 µl/ml. The lack of a good dose response over the high end of the dose range and the complete absence of a response with mouse liver S9 mix places the weak response under some suspicion. The compound could be considered only weakly active at best.

Submitted by:

Study Director

Dale W. Matheson, Ph.D. — Date 7/10/78
Associate Director and
Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:

David Brusick, Ph.D. — Date 7/10/78
Director
Department of Genetics
and Cell Biology

TABLE 1

4. RESULTS

LBI DATA REPORT SHEET: UNSCHEDULED DNA SYNTHESIS IN WI-38 CELLS

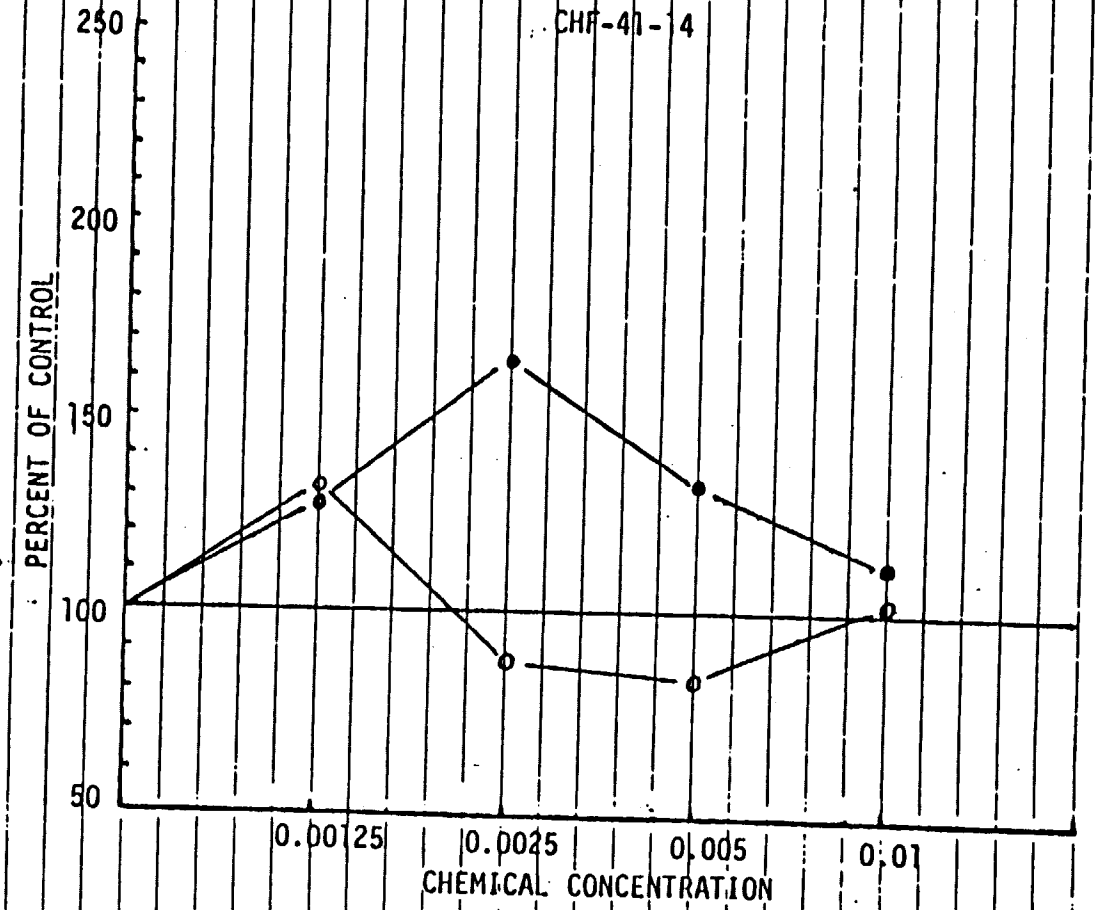
CLIENT: Carnegie-Mellon CHEMICAL: CHF-41-14

ASSAY#: 2827 DATE OF TEST INITIATION: May 31, 1978

SOLVENT: DMSO

TEST	COMPOUND CONCENTRATION	DNA UG	DPM	DPM UG/DNA	% OF CONTROL
NONACTIVATION					
Solvent Control	--	19.14	887	46	100
Positive Control (MNNG)	5 µg/ml	20.13	3496	174	378
Test Compound					
CHF-41-14	0.00125 µl/ml	17.82	1057	59	128
CHF-41-14	0.0025 µl/ml	19.96	1516	76	165
CHF-41-14	0.005 µl/ml	7.10	433	61	133
CHF-41-14	0.01 µl/ml	20.96	1075	51	111
ACTIVATION					
Solvent Control	--	12.38	555	45	100
Positive Control (BαP)	2.5 µg/ml	23.60	1908	81	180
Test Compound					
CHF-41-14	0.00125 µl/ml	15.18	904	60	133
CHF-41-14	0.0025 µl/ml	13.53	531	39	87
CHF-41-14	0.005 µl/ml	5.78	217	38	84
CHF-41-14	0.01 µl/ml	22.77	1074	47	104

TABLE 2
INCORPORATION OF ^{3}H-THYMIDINE
IN WI-38 CELLS
CHF-41-14



PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for its ability to induce unscheduled DNA synthesis (UDS) in human diploid WI-38 cells blocked in G_1 phase.

2. MATERIALS

A. Indicator Cells

Diploid WI-38 cells derived from human embryonic lung tissue were used from a commercial supplier.

B. Media

Growth medium (GM) consisted of Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum (FCS) and penicillin-streptomycin (PS).

Maintenance medium (SM) consisted of EMEM supplemented with 0.5% FCS and PS.

Hydroxyurea medium (HUM) consisted of SM plus hydroxyurea to a final concentration of 10^{-2} M.

C. Control Compounds

1. Negative Control

The material used as the solvent for the test chemical was used as the negative control. The solvent is listed in Table 1 in Section V. Results. The volume of solvent in the negative control test was equal to the high amount of solvent added with the test chemical.

2. Positive Control

N-methyl nitrosoguanidine (MNNG) at a concentration of 5 µg/ml was used as the positive control agent in nonactivation tests, and Benzo(a)pyrene (BaP) at a concentration of 10 µg/ml was used as the positive control agent in the activation tests.

3. EXPERIMENTAL DESIGN

A. Cell Preparation

Normal human diploid WI-38 cells were seeded at 5 x 10^5 cells in a 100 mm tissue culture dish and grown to confluency in GM. Once reaching confluency, the cells were switched to SM for 5 days. The contact inhibition imposed by confluency, and the use of SM, held the cells in a nonproliferating state.

B. Treatment

On the day of treatment, cells held in G_1 phase were placed in HUM. After 30 min, this medium was replaced by 2 ml HUM containing the control or test chemical, and 1.0 µCi ^{3}HTdR. An assay consisted of at least 3 concentrations. Exposure was terminated after 1.5 hr by washing the cells twice in a cold balanced salt solution (BSS) containing an excess of cold thymidine. The test concentrations were selected from a series of standard concentrations ranging from 0.1 µg/ml to 5.0 µg/ml. A lower series was used if all standard concentrations proved to be toxic.

Treated plates were frozen at -20°C until processed. After thawing, the cells on the 100 mm plate were covered with 0.1% sodium dodecyl sulfate (SDS) in (SSC) (0.15M sodium chloride-0.015M sodium citrate) and scraped from the dish with a rubber spatula. The cells were washed and precipitated from the SDS by 3 changes of 95% ethanol and centrifuged at 10,000 x g. Additional lipid components were removed by extraction in ethanol ether at 70°C. This pellet was washed in 70% ethanol, further incubated at 70°C in 0.3N NaOH, and the DNA extracted in 1000 µl 1N perchloric acid (PCA) at 70 C. The DNA was separated into two 500 µl aliquots. One of these was solubilized in 10 ml scintillation cocktail and counted in a Packard liquid scintillation spectrometer. The second aliquot was read at 260 nm in a UV spectrophotometer. The values were corrected for light scatter and converted to micrograms DNA. Following liquid scintillation counting, the data were combined with the DNA extraction values and expressed as disintegrations per minute per microgram DNA (DPM/µg DNA).

D. Activation System

Because metabolic activation is essential for the expression of biological activity in some chemicals, a liver activation system containing an Aroclor 1254 induced hepatic S9 fraction was employed. The activation system consisted of:

3. EXPERIMENTAL DESIGN (Continued)

D. Activation System

Component	Final Concentration/ml
NADP (sodium salt)	6 µmol
Isocitric acid	35 µmol
Tris buffer, pH 7.4	28 µmol
$MgCl_2$	2 µmol
Mouse liver S9 (9,000 x g)	100 µl

4. EVALUATION CRITERIA

Several criteria have been established which, if met, provide a basis for declaring a material genetically active in the UDS assay. These criteria are derived from a historical data base and are helpful in maintaining uniformity in evaluations from material to material and run to run. While these criteria are reasonably objective, a certain amount of flexibility may be required in making the final evaluations since absolute criteria may not be applicable to all biological data.

A compound is considered active in the UDS assay if:

a. A dose-response relationship is observed over two consecutive dose levels.

b. The minimum increase at the high level of the dose response is approximately 150% of the control value. The positive control data for a large sampling 5 µg/ml of MNNG tests was found to be 206% of the control and for 10 µg/ml of Benz(a)pyrene it was 162% of the control.

All evaluations of UDS activity are based on the concurrent solvent control value run with the experiment in question. Positive control values are not used as reference points to measure activity, but rather to demonstrate that the cell population employed was responsive to chemicals known to induce repair synthesis under the appropriate test conditions.

As the data base for the UDS assay increases, the evaluation criteria will be more firmly established.

STANDARD OPERATING PROCEDURE

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

AFDR

MUTAGENICITY EVALUATION OF

CHF-41-14 — UCC-A186

IN THE
MOUSE LYMPHOMA FORWARD
MUTATION ASSAY

FINAL REPORT

SUBMITTED TO:

CARNEGIE-MELLON
4400 FIFTH AVENUE
PITTSBURGH, PA 15213

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20839

JUNE 1978

Litton BIONETICS

PREFACE

This report contains a summary of the data compiled during the evaluation of the test material. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I - IV and provides sponsor and compound identification information, type of assay and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V identifies the tables and/or figures containing the data used by the Study Director in interpreting the test results (item VI).

The second part of the report, entitled PROTOCOL, describes, in detail, the materials and procedures employed in conducting the evaluation. This part of the report also contains evaluation criteria, standard operating procedures and any appendices. These are included to acquaint the sponsor with the methods used to develop and analyze the test results. All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology. Copies of raw data will be supplied to the sponsor upon request.

I. SPONSOR: Carnegie-Mellon

II. MATERIAL*

A. Identification: CHF-41-14

B. Date Received: February 7, 1978

C. Physical Description: Pale yellow liquid

III. TYPE OF ASSAY: Mouse Lymphoma Forward Mutation Assay

IV. PROTOCOL NO.: DMT-106

V. RESULTS

The data presented in Table 1 show the concentrations of the test compound employed, number of mutant clones obtained, surviving populations after the expression period, and calculated mutation frequencies. All calculations are performed by computer program.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was prepared as a stock in DMSO (250 µl/ml) and was diluted by two-fold steps to establish a series of concentrations for toxicity testing. The compound was examined for its cytotoxic potential over a series of concentrations ranging from 0.32 µl/ml to 2.5 µl/ml. The concentrations employed in the actual test were selected from this series and consisted of levels extending from nontoxicity to levels showing some evidence of compound induced cytotoxicity. The concentrations employed in the test are shown in Table 1.

CHF-41-14 was evaluated for mutagenic activity at the TK locus of L5178Y mouse lymphoma cells. A series of concentrations selected on the basis of preliminary toxicity tests were employed and produced sufficient toxicity for a reliable study.

*Information was supplied by the sponsor. If information was not indicated by the sponsor, N.I. was entered.

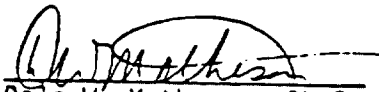
VI. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

The material was shown to be a mutagen in this assay and produced a dose related increase in mutation frequency under nonactivation (-S9) conditions. The data from the activation tests were also elevated at the higher concentration levels but the magnitude of the responses was much less than that observed without S9. This information could be viewed as an indication of quenching or detoxification by the S9 fraction.

The test material, CHF-41-14, was mutagenic in the Mouse Lymphoma Forward Mutation Assay. The mutagenic response was greater under nonactivation test conditions than activation test conditions.

Submitted by:

Study Director

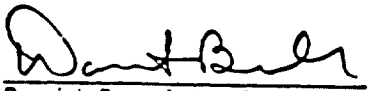


6/2/78

Dale W. Matheson, Ph.D.
Associate Director and
Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

Date

Reviewed by:



6/2/78

David Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

Date

TABLE 1

4a. SUMMARY OF MOUSE LYMPHOMA ASSAY RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: CHF-41-14
B. SOLVENT: DMSO
C. TEST DATE: 05/08/78
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) OR NANOLITERS(NL) PER MILLILITER.

TEST		S-9 SOURCE	S-9 TISSUE	DAILY COUNTS CELLS/ML X 10E5 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** X 10E-6
NONACTIVATION												
SOLVENT CONTROL		---	---	15.1	13.3	15.3	100.0	46.0	227.0	100.0	100.0	20.0
NEGATIVE CONTROL		---	---	16.8	12.4	10.2	69.2	21.0	342.0	150.7	104.2	6.1
EMS 0.5UL/ML		---	---	10.8	9.2	10.8	34.9	716.0	200.0	88.1	30.8	358.0
TEST COMPOUND												
0.640	UL/ML	---	---	14.6	12.4	13.0	76.6	66.0	263.0	115.9	88.7	25.1
1.250	UL/ML	---	---	11.8	9.6	8.2	30.2	143.0	305.0	134.4	40.6	46.9
1.500	UL/ML	---	---	10.6	12.2	7.6	32.0	126.0	305.0	134.4	43.0	41.3
1.700	UL/ML	---	---	8.2	10.8	7.6	21.9	130.0	201.0	88.5	19.4	64.7
2.000	UL/ML	---	---	0.6	2.8	7.8	2.3	247.0	170.0	74.9	1.7	145.3
ACTIVATION												
SOLVENT CONTROL		RAT	LIVER	4.5	11.0	11.8	100.0	57.0	294.5	100.0	100.0	19.0
NEGATIVE CONTROL		RAT	LIVER	7.4	11.2	12.0	170.3	41.0	317.0	107.6	183.3	12.9
DMN .3UL/ML		RAT	LIVER	5.2	4.8	2.8	12.0	148.0	8.0	2.7	0.3	1850.0
TEST COMPOUND												
0.020	UL/ML	RAT	LIVER	3.6	12.0	9.8	61.2	80.0	327.0	111.0	67.9	24.5
0.040	UL/ML	RAT	LIVER	2.0	4.4	12.6	28.5	95.0	239.0	81.2	23.1	39.7
0.080	UL/ML	RAT	LIVER	0.8	4.4	8.4	19.5	174.0	293.0	99.5	19.4	59.4
0.160	UL/ML	RAT	LIVER	3.4	7.6	7.6	29.3	132.0	335.0	113.8	33.3	39.4

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** (MUTANT CLONES / VIABLE CLONES) X 10E-6

PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for specific locus forward mutation induction in the L5178Y thymidine kinase (TK) mouse lymphoma cell assay.

2. MATERIALS

A. Indicator Cells

The cells used in this study were derived from Fischer mouse lymphoma cell line L1578Y. The cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine BrdU sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BrdU.

B. Media

The cells were maintained in Fischer's medium for leukemic cells of mice with 10% horse serum and sodium pyruvate. Cloning medium consisted of Fischer's medium with 20% horse serum, sodium pyruvate, and 0.3% Noble agar. Selection medium was made from cloning medium by the addition of 7.5 mg BrdU to 100 ml cloning medium.

C. Control Compounds

1. Negative Control

The solvent in which the test compound was dissolved was used as a negative control and is designated as solvent control in the data table. The actual solvent is listed in Table 1 of Section V Results.

2. Positive Control

Ethylmethanesulfonate (EMS), which induces mutation by base-pair substitution, was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of 0.5 µl/ml.

Dimethylnitrosamine (DMN), which induces mutation by base-pair substitution and requires metabolic biotransformation by microsomal enzymes, was used as a positive control substance for the activation studies at a final concentration of 0.5 µl/ml.

3. EXPERIMENTAL DESIGN

A. Toxicity

The solubility, toxicity, and doses for all chemicals were determined prior to screening. The effect of each chemical on the survival of the indicator cells was determined by exposing the cells to a wide range of chemical concentrations in complete growth medium. Toxicity was measured as loss in growth potential of cells induced by a 4-hr exposure to the chemical followed by a 24-hr expression period in growth medium. A minimum of 4 concentrations was selected from the concentration range by using as the highest dose a level that showed a reduction in growth potential. At least 3 lower doses, including levels which were below the toxic range, were added. Those compounds that were relatively nontoxic to cells growing in suspension were tested at concentrations of up to 10 mg/ml when solubility permitted. Toxicity produced by chemical treatment was monitored during the experiment.

B. Assays

1. Nonactivation Assay

The procedure used is a modification of that reported by Clive and Spector (1975). Prior to each treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and glycine (THMG). This medium permits the survival of only those cells that produce TK, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium at the predetermined doses for 4 hr at 37 C on a rocker. The treated cells were washed, fed, and allowed to express in growth medium for 3 days. At the end of this expression period. TK-/- mutants were detected by cloning the cells in the selection medium for 10 days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium.

2. Activation Assay

The activation assay differs from the nonactivation assay in the following manner only. S9 was added to 10 milliliters growth medium containing appropriate cofactors and the desired number of cleansed cells. After adding the test compound, the flask was incubated with agitation for 4 hr at 37 C. The incubation period was terminated by washing the cells twice with growth medium. The washed treated cells were then allowed to express for 3 days and were cloned as indicated for the nonactivation cells.

3. EXPERIMENTAL DESIGN (Continued)

C. Preparation of 9,000 x g Supernatant

Male, random bred rats (Fischer 344) pretreated with Aroclor 1254 were killed by cranial blow, decapitated, and bled. The liver was immediately dissected from the animal using aseptic technique and placed in ice-cold 0.25 M sucrose buffered with Sodium Phosphate at pH 7.4. When an adequate number of livers had been collected, they were washed twice with fresh buffered sucrose and completely homogenized. The homogenate was centrifuged for 20 min at 9,000 x g in a refrigerated centrifuge. The supernatant from this centrifuged sample was retained and frozen at -80 C until used in the activation system. This microsome preparation was added to culture medium along with the appropriate cofactors in the concentrations described below:

Component	Final Concentration/ml
NADP (sodium salt)	2.4 mg
Isocitric acid	4.5 mg
Homogenate S9 fraction	10 µl

D. Screening

A mutation index was derived by dividing the number of clones formed in the BrdU-containing selection medium by the number found in the same medium without BrdU. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. Colonies were counted on an electronic colony counter that resolves all colonies greater than 200 microns in diameter.

4. EVALUATION CRITERIA

Several criteria have been established which, if met, provide a basis for declaring a material genetically active in the Mouse Lymphoma Forward Mutation Assay. These criteria are derived from a historical data base and are helpful in maintaining uniformity in evaluations from material to material, and run to run. While these criteria are reasonably objective, a certain amount of flexicility may be required in making the final evaluation since absolute criteria may not be applicable to all biological data.

4. EVALUATION CRITERIA (Continued)

A compound is considered mutagenic in this assay if:

- A dose-response relationship is observed over 3 of the 4 dose levels employed.
- The minimum increase at the high level of the dose-response curve is at least 2.5 times greater than the solvent and/or negative control values.
- The solvent and negative control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on consideration of the concurrent solvent and negative control values run with the experiment in question. Positive control values are not used as reference points, but are included to ensure that the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

As the data base on the assay increases, the evaluation criteria can be expected to become more firmly established.

REFERENCES

Clive, D. and Spector, J.F.S. (1975). Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Res. 31, 17-29.

5. STANDARD OPERATING PROCEDURE

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

William C. Kuryla, Ph.D.
Associate Director, Product Safety
Union Carbide Corporation
39 Old Ridgebury Road
Danbury, Connecticut 06817-0001

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

FEB 27 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and **refer to the reverse side of this page for "EPA Information Requests"** .

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

12832A

Triage of 8(e) Submissions

Date sent to triage: FEB 2 4 1995

NON-CAP (CAP)

Submission number: 12832 A

TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX (GTOX)

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: (0) 1 2 **pages** 1,2 **pages** 1,2, tabs

Notes:

Contractor reviewer : LPS **Date:** 1/5/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ 0892 - 12832 SEQ A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Union Carbide Corporation

SUB. DATE: 08/21/92 OTS DATE: 08/28/92 CSRAD DATE: 09/29/94

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: Silane A-186

CAS# 3388-04-3

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 (02) 04
(0204)	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
(0224)	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
(0244)	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
(0247)	DNA DAM/REPAIR	01 (02) 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	(YES)	YES (DROP/REFER)	In Vitro	LOW		
CAS SR	NO	NO (CONTINUE)		(MED)		
	DETERMINE	REFER:		HIGH		

COMMENTS: 8ehq - 0681 - 0402

11) 8EHQ-92-12832: Rank - medium.

Chemical: Silane A-186 (CAS# 3388-04-3).

Mutagenicity evaluation of CHF-40-321, Litton Bionetics, Kensington MD, dated November 1977: Positive for gene mutations in the Salmonella typhimurium/mammalian microsomal (Ames) assay in strains TA100 and TA1535 both without and with metabolic activation.

Mutagenicity evaluation of CHF-40-321 in the mouse lymphoma forward mutation assay, Litton Bionetics, Kensington MD, dated June 1976: Positive for gene mutations in the L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay in vitro both without and with metabolic activation.

Mutagenicity evaluation of CHF-41-14 in the sister chromatid exchange assay in L5178Y mouse lymphoma cells, Litton Bionetics, Kensington MD, dated March 1979: Induces DNA effects in the form of sister chromatid exchanges (SCEs) in L5178Y mouse lymphoma cells in vitro both without and with metabolic activation.

Mutagenicity evaluation of CHF-41-14 in the unscheduled DNA synthesis in human WI-38 cells assay, Litton Bionetics, Kensington MD, dated July 1978: Induces DNA effects (weakly) in the form of unscheduled DNA synthesis (UDS) in human WI-38 cells in vitro without but not with metabolic activation.